ADAGYAPATPAAAGAAAGKATTEEQKLIEDINVGFKAAVAAAASVPAADK

1 10 20 30 40 50

FKTFEAAFTSSSKAAAAKAPGLVPKLDAAYSVAYKAAVGATPEAKFDSFV

51 60 70 80 90 100

ASLTEALRVIAGALEVHAVKPVTEEPGMAKIPAGELQIIDKIDAAFKVAA

101 110 120 130 140 150

TAAATAPADDKFTVFEAAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAA

151 160 170 180 190 200

TVAAAPQVKYAVFEAALTKAITAMSEVQKVSQPATGAATVAAGAATTAAG

201 210 220 230 240 250

AASGAATVAAGGYKV (SEQ ID NO 87)

251 260 265

The invention particularly relates to modified recombinant allergens in which at least one, or a combination, of the regions 16-42, 135-149 and 180-206 of the Phl p 5b polypeptide, consisting of a total of 265 amino acids, is/are not altered. The segments to be preserved are the T cell epitope regions.

The said amino acid residues can also be derivatized. Modifications of the side chains are particularly appropriate in this context.

The amino acid residue abbreviations which are listed above and below stand for the residues of the following amino acids:

Ala = A alanine
Asn = N asparagine
Asp = D aspartic acid
Arg = R arginine
Cys = C cysteine
Gln = Q glutamine
Glu = E glutamic acid
Gly = G glycine

In this context, PM1 denotes point mutation 1 and has the following sequence (the amino acids which are replaced as compared with Phl p 5b are printed in bold):

ADAGYAPATPAAAGAAAGKATTEEQKLIEDI**D**VGFKAAVAAAASVPAA**LA**

1 10 20 30 40 50

FKTFEAAFTSSSKAAAAKAPGLVPKLDAAYSVAYKAAVGATPEAKFDSFV

51 60 70 80 90 100

ASLTEALRVIAGALEVHAVKPVTEEPGMAKIPAGELQIIDKIDAAFKVAA

101 110 120 130 140 150

TAAATAPADDKFTVFEAAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAA

151 160 170 180 190 200

TVAAAPQVKYAVFEAALTKAITAMSEVQKVSQPATGAATVAAGAATTAAG

201 210 220 230 240 250

AASGAATVAAGGYKV (SEQ ID NO 88)

251 260 265

The other particularly preferred peptides have the following sequences:

PM2 ($D^{49} \rightarrow L$, $K^{50} \rightarrow A$):

ADAGYAPATPAAAGAAAGKATTEEQKLIEDI**N**VGFKAAVAAAASVPAA**LA**

1 10 20 30 40 50

FKTFEAAFTSSSKAAAAKAPGLVPKLDAAYSVAYKAAVGATPEAKFDSFV

51 60 70 80 90 100

ASLTEALRVIAGALEVHAVKPVTEEPGMAKIPAGELQIIDKIDAAFKVAA

101 110 120 130 140 150

TAAATAPADDKFTVFEAAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAA

151 160 170 180 190 200

TVAAAPQVKYAVFEAALTKAITAMSEVQKVSQPATGAATVAAGAATTAAG

201 210 220 230 240 250

AASGAATVAAGGYKV (SEQ ID NO 89)

251 260 265

PM3 ($A^{13} \rightarrow C$):

ADAGYAPATPAACGAAAGKATTEEQKLIEDINVGFKAAVAAAASVPAADK

1 10 20 30 40 50

FKTFEAAFTSSSKAAAAKAPGLVPKLDAAYSVAYKAAVGATPEAKFDSFV

51 60 70 80 90 100

ASLTEALRVIAGALEVHAVKPVTEEPGMAKIPAGELQIIDKIDAAFKVAA

101 110 120 130 140 150

TAAATAPADDKFTVFEAAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYAA

151 160 170 180 190 200

TVAAAPQVKYAVFEAALTKAITAMSEVQKVSQPATGAATVAAGAATTAAG

201 210 220 230 240 250

AASGAATVAAGGYKV (SEQ ID NO 90)

251 260 265

DM1 ($\Delta\ K^{50} \rightarrow P^{\Delta 132}$, $D^{49} \rightarrow L$):

ADAGYAPATPAAAGAAAGKATTEEQKLIEDINVGFKAAVAAAASVPAALA

1 10 20 30 40 50

GELQIIDKIDAAFKVAATAAATAPADDKFTVFEAAFNKAIKESTGGAYDTYK

51 60 70 80 90 100

CIPSLEAAVKQAYAATVAAAPQVKYAVFEAALTKAITAMSEVQKVSQPATG

103 110 120 130 140 150

AATVAAGAATTAAGAASGAATVAAGGYKV (SEQ ID NO 91)

154 160 170 180

DM 2 ($\Delta\ F^{51}$ - G^{178}, D^{49} - L, K^{50} - A):

ADAGYAPATPAAAGAAAGKATTEEQKLIEDINVGFKAAVAAAASVPAALA

1 10 20 30 40 50

GAYDTYKCIPSLEAAVKQAYAATVAAAPQVKYAVFEAALTKAITAMSEVQK

51 60 70 80 90 100

VSQPATGAATVAAGAATTAAGAASGAATVAAGGYKV (SEQ ID NO 92)

102 110 120 130 137

DM2* (Δ F^{51} - G^{178}, 179 - 217 altered sequence):

This sequence corresponds to that of DM2 where, however, the amino acids of positions 179 - 217 of the starting peptide Phl p 5b additionally exhibit an altered sequence and all the subsequent amino acids are missing.

DM3 (Δ A^{154} - T^{177}, $A^{220} \rightarrow T$):

ADAGYAPATPAAAGAAAGKATTEEQKLIEDINVGFKAAVAAAASVPAADK

1 10 20 30 40 50

FKTFEAAFTSSSKAAAAKAPGLVPKLDAAYSVAYKAAVGATPEAKFDSFV

51 60 70 80 90 100

ASLTEALRVIAGALEVHAVKPVTEEPGMAKIPAGELQIIDKIDAAFKVAA

101 110 120 130 140 150

TAAGGAYDTYKCIPSLEAAVKQAYAATVAAAPQVKYAVFEAALTKTITAMS

151 160 170 180 190 200

EVQKVSQPATGAATVAAGAATTAAGAASGAATVAAGGYKV (SEQ ID NO 93)

202 210 220 230 240

The invention furthermore relates to a process for preparing modified recombinant allergens by using the polymerase chain reaction and/or its variants. When the peptide sequence is known, the allergens can also be prepared by means of methods of peptide synthesis which are known per se, e.g. the modified Merrifield technique, as described in the literature (e.g. in the standard works such as Houben-Weyl, Methoden der organischen Chemie (Methods of Organic Chemistry), Georg-Thieme-Verlag, Stuttgart;), under reaction conditions which are known and are suitable for the said reactions. In this context, use can also be made of variants which are known per se but which are not mentioned here in detail. It is furthermore possible to liberate the peptides from one of their functional

present in allergic patients and to exert a therapeutic influence on these cells.

Tab. 1: Dodecapeptides which are based on the Phl p 5b sequence and which are used for determining the T cell-reactive regions

1	ADAGYAPATPAA	(SEQ ID NO 1)	44	KIPAGELQIIDK	(SEQ ID NO 44)
2	GYAPATPAAAGA	(SEQ ID NO 2)	45	AGELQIIDKIDA	(SEQ ID NO 45)
3	PATPAAAGAAAG	(SEQ ID NO 3)	46	LQIIDKIDAAFK	(SEQ ID NO 46)
4	PAAAGAAAGKAT	(SEQ ID NO 4)	47	IDKIDAAFKVAA	(SEQ ID NO 47)
5	AGAAAGKATTEE	(SEQ ID NO 5)	48	IDAAFKVAATAA	(SEQ ID NO 48)
6	AAGKATTEEQKL	(SEQ ID NO 6)	49	AFKVAATAAATA	(SEQ ID NO 49)
7	KATTEEQKLIED	(SEQ ID NO 7)	50	VAATAAATAPAD	(SEQ ID NO 50)
8	TEEQKLIEDINV	(SEQ ID NO 8)	51	TAAATAPADDKF	(SEQ ID NO 51)
9	QKLIEDINVGFK	(SEQ ID NO 9)	52	ATAPADDKFTVF	(SEQ ID NO 52)
10	IEDINVGFKAAV	(SEQ ID NO 10)	53	PADDKFTVFEAA	(SEQ ID NO 53)
11	INVGFKAAVAAA	(SEQ ID NO 11)	54	DKFTVFEAAFNK	(SEQ ID NO 54)
12	GFKAAVAAAASV	(SEQ ID NO 12)	55	TVFEAAFNKAIK	(SEQ ID NO 55)
13	AAVAAAASVPAA	(SEQ ID NO 13)	56	EAAFNKAIKEST	(SEQ ID NO 56)
14	AAAASVPAADKF	(SEQ ID NO 14)	57	FNKAIKESTGGA	(SEQ ID NO 57)
15	ASVPAADKFKTF	(SEQ ID NO 15)	58	AIKESTGGAYDT	(SEQ ID NO 58)
16	PAADKFKTFEAA	(SEQ ID NO 16)	59	ESTGGAYDTYKC	(SEQ ID NO 59)
17	DKFKTFEAAFTS	(SEQ ID NO 17)	60	GGAYDTYKCIPS	(SEQ ID NO 60)
18	KTFEAAFTSSSK	(SEQ ID NO 18)	61	YDTYKCIPSLEA	(SEQ ID NO 61)
19	EAAFTSSSKAAA	(SEQ ID NO 19)	62	YKCIPSLEAAVK	(SEQ ID NO 62)
20	FTSSSKAAAAKA	(SEQ ID NO 20)	63	IPSLEAAVKQAY	(SEQ ID NO 63)
21	SSKAAAAKAPGL	(SEQ ID NO 21)	64	LEAAVKQAYAAT	(SEQ ID NO 64)
22	AAAAKAPGLVPK	(SEQ ID NO 22)	65	AVKQYAATYAA	(SEQ ID NO 65)
23	AKAPGLVPKLDA	(SEQ ID NO 23)	66	QAYAATVAAAPQ	(SEQ ID NO 66)
24	PGLVPKLDAAYS	(SEQ ID NO 24)	67	AATVAAAPQVKY	(SEQ ID NO 67)
25	VPKLDAAYSVAY	(SEQ ID NO 25)	68	VAAAPQVKYAVF	(SEQ ID NO 68)
26	LDAAYSVAYKAA	(SEQ ID NO 26)	69	APQVKYAVFEAA	(SEQ ID NO 69)
27	AYSVAYKAAVGA	(SEQ ID NO 27)	70	VKYAVFEAALTK	(SEQ ID NO 70)
28	VAYKAAVGATPE	(SEQ ID NO 28)	71	AVFEAALTKAIT	(SEQ ID NO 71)
29	KAAVGATPEAKF	(SEQ ID NO 29)	72	EAALTKAITAMS	(SEQ ID NO 72)
30	VGATPEAKFDSF	(SEQ ID NO 30)	73	LTKAITAMSEVQ	(SEQ ID NO 73)
31	TPEAKFDSFVAS	(SEQ ID NO 31)	74	AITAMSEVQKVS	(SEQ ID NO 74)
32	AKFDSFVASLTE	(SEQ ID NO 32)	75	AMSEVQKVSQPA	(SEQ ID NO 75)
33	DSFVASLTEALR	(SEQ ID NO 33)	76	EVQKVSQPATGA	(SEQ ID NO 76)
34	VASLTEALRVIA	(SEQ ID NO 34)	77	KVSQPATGAATV	(SEQ ID NO 77)
35	LTEALRVIAGAL	(SEQ ID NO 35)	78	QPATGAATVAAG	(SEQ ID NO 78)
36	ALRVIAGALEVH	(SEQ ID NO 36)	79	TGAATVAAGAAT	(SEQ ID NO 79)
37	VIAGALEVHAVK	(SEQ ID NO 37)	80	ATVAAGAATTAA	(SEQ ID NO 80)
38	GALEVHAVKPVT	(SEQ ID NO 38)	81	AAGAATTAAGAA	(SEQ ID NO 81)
39	EVHAVKPVTEEP	(SEQ ID NO 39)	82	AATTAAGAASGA	(SEQ ID NO 82)
40	AVKPVTEEPGMA	(SEQ ID NO 40)	83	TAAGAASGAATV	(SEQ ID NO 83)
41	PVTEEPGMAKIP	(SEQ ID NO 41)	84	GAASGAATVAAG	(SEQ ID NO 84)
42	EEPGMAKIPAGE	(SEQ ID NO 42)	85	SGAATVAAGGYK	(SEQ ID NO 85)
43	GMAKIPAGELOI	(SEQ ID NO 43)	86	GAATVAAGGYKV	(SEQ ID NO 86)

Literature:

1. Müller WD, Karamfilov T, Fahlbusch B, Vogelsang H, Jäger L:
"Analysis of human T cell clones reactive with group V grass pollen allergens". Int. Arch. Allergy Immunol. 1994, 105:391-396.

2. Jung K, Fahlbusch B, Müller WD, Hermann D, Diener C, Jäger L:
"Isolation of timothy (Phleum pratense) allergens using affinity chromatography with monoclonal antibodies". Allergy Immunol (Leipzig) 1989, 35:287-294.

3. Bufe A, Schramm G, Keown MB, Schlaak M, Becker WM:
"Major allergen Phl p 5b in timothy grass is a novel pollen Rnase". FEBS Letters 1995, 263:6-12.

Example 2

Preparation of point mutants PM1, PM2 ($D^{48} \rightarrow L$, $K^{50} \rightarrow A$) and PM3 ($A^{13} \rightarrow C$) of rPhl p 5b

PM2:

Plasmid pGS13 was used as the starting vector. This is the pMalc vector (Biolabs) which contains the cDNA for the wt rPhl p 5b which is cloned between Bam HI and Hind III sites. Fragments 1 (bp: 1 - 153) and 2 (bp: 141 - 1374) of the cDNA for the rPhl p 5b were amplified in a PCR reaction. The following primers (restriction sites are underlined) were used for this reaction:

Fragment 1:

Phl p 5b sense:
5'-ATATGGATCCATCGAGGGAAGGGCCGATGCCGGCTACGCC-3' (SEQ ID NO 94)

MP1 antisense:
5'-GAACGCTAGCGCCGCAGGGACGCTGGC-3' (SEQ ID NO 95)

Fragment 2:

MP1 sense:
5'-GCGCTAGCGTTCAAGACCTTCGAG-3' (SEQ ID NO 96)

Phl p 5b antisense:
5'-ATATAAGCTTTCCTCTGAAGGAAGGCAACCC-3' (SEQ ID NO 97)

As compared with the wt sequence, the two mutagenesis primers MP1 sense and MP1 antisense contain 6 base replacements which additionally give rise to a new restriction cleavage site for the enzyme Nhe I.

The amplified fragment 1 was digested with Bam HI and Nhe I and cloned into vector pUH89 (Jekel et al., Gene: 154, 55-59; 1995). The resulting plasmid, pGS10, was restricted once again with Nhe I/Hind III, and fragment 2 (Nhe I/Hind III) was incorporated into these cleavage sites. This plasmid, pGS11, comprises the complete cDNA encoding rPhl p 5b but containing the desired base replacements. In order to express the point mutant rPhl p 5b PM2, the mutated cDNA was recloned between the Bam HI and Hind III cleavage sites of the expression vector pMalc. The resulting plasmid was designated pGS21.

The point mutant rPhl p 5b PM1 was prepared in analogy with PM2. It contains, as the result of a PCR error, an additional point mutation: $N^{32} \rightarrow D$.
In order to clone this point mutant, the entire cDNA for rPhl p 5b in vector pGS13 was amplified in a PCR using the following primers.

PCysM1:
5'ATATGGATCCATCGAGGGTAGGGCCGATGCCGGCTACGCCCCGGC
CACCCCGGCTGCATGCGGAGCG-3' (SEQ ID NO 98)

Phl p 5b antisense: see above.

As compared with the wt sequence, the mutagenesis primer PCysM1 contains 3 base substitutions which lead to an alanine residue being replaced with a cysteine residue and which at the same time give rise to a new restriction cleavage site for the enzyme Sph I. The PCR product was cloned directly into the pMalc expression vector (Bam HI/Hind III). The resulting vector was designated pCysM1. The success of the mutagenesis was checked in a restriction analysis using Sph I.

Example 3

Preparation of the deletion mutants DM1 (ΔK^{50} - P^{132}, $D^{49} \rightarrow L$), DM2 (ΔF^{51} - G^{178}, $D^{49} \rightarrow L$, $K^{50} \rightarrow A$) and DM3 (ΔA^{154} - T^{177}, $A^{220} \rightarrow T$)

Plasmid pGS21 (see above) was used as the starting vector for cloning the deletion mutant DM1. The bp 399 - 1374 fragment of the cDNA for rPhl p 5b was amplified in a PCR using the following primers:

MP2 sense:
5'-GCTAGCCGGCGAGCTGCAGATCATCG-3' (SEQ ID NO 99)

Phl p 5b antisense: see above.

Vector pGS21 was restricted with Nhe I and Bam HI and separated from the excised fragment. The PCR product, which had also been restricted with Nhe I and Bam HI, was then ligated into the residual vector. The vector which resulted from this, i.e. pDM1, contains the rPhl p 5b cDNA which has a deletion of 252 bp and which encodes the deletion mutant rPhl p 5bDM1. Deletion mutants DM2 and DM3 were prepared in an analogous manner.